

Structure and evolution of spiracular gills in pupae of net-winged midges (Nematocera; Blephariceridae): Part III: Gill diversity in *Paulianina* (subfamily Edwardsiniinae)

by

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ABSTRACT

The pupal gill structure of seven species of the Madagascan genus *Paulianina* has been examined by scanning electron microscopy and compared with that of other Edwardsiniinae. The respiratory organs of *P. ingens* (subgenus *Paulianina* s.s.) have a primitive anatomy and resemble in some respects the gills of the neotropic *Edwardsina chilota*, whereas the species of the subgenus *Eupaulianina* are unique among Blephariceridae not only because of the peculiar shape of their respiratory processes, but also because of the special structure of the plastron. An additional apomorphic character of this subgenus is a blister at the terminal end of the dorsal granules on the pupal tergites. *P. umbra* (*Paulianina* s.s.) seems to be an intermediate form with regard to gill structure. Based on the morphological findings, a phylogeny of *Paulianina* is proposed that includes, contrary to current systematics, the subgenus *Paulianina* s.s. as a paraphyletic taxon. The adaptive value of the transformation of respiratory lamellae into broad swellings is obscure, because all *Paulianina* species develop in fast-flowing streams and therefore live in the same type of habitat as other blepharicerids. The complex pattern of arrangement of the dorsal granules of *Eupaulianina* agrees well with the flow pattern around blepharicerid pupae.

INTRODUCTION

In the context of an extensive investigation on spiracular gills in blepharicerid pupae, I have previously presented two studies on species of the Edwardsiniinae. In the first publication (Arens 1995), conclusions regarding the primitive gill structure in this subfamily were drawn by comparison of species from the three geographically isolated faunal entities of the taxon (Australia, South America, Madagascar), whereas the second study was concerned with the amazing diversity of gill structure among Australian *Edwardsina* (Arens 1998). Because of the generosity of Brian R. Stuckenberg, I have recently also had the unexpected opportunity to examine, by scanning electron microscopy (SEM), the respiratory organs of the Madagascan genus *Paulianina*. This genus consists of two subgenera (*Paulianina* s.s. and *Eupaulianina*) that differ considerably from one another in pupal gill shape and other features. Only 10 *Paulianina* species have been described to date; presumably others live on Madagascar (Stuckenberg 1958), providing that they have not become extinct in the meantime.

Information concerning the gills of *Paulianina* as obtained by SEM is limited to four micrographs in Hinton's review of spiracular gills (Hinton 1968), and to my description of *Paulianina umbra*, a species of *Paulianina* s.s. (Arens 1995). Another species of this subgenus, *P. ingens*, has been examined in the present study. The fine structure of the peculiarly shaped gills of the seven *Eupaulianina* species is

completely unknown, for Stuckenberg had no scanning electron microscope at his disposal for his excellent monograph of the genus (Stuckenberg 1958). I have now investigated *P. pamela*, *P. rivalis*, *P. silva* and *P. alexanderi*, so that only one of the *Paulianina* species with known pupae (*P. spec. I*) is not included in my study.

Because of the ecological devastation of Madagascar, it is feared that some *Paulianina* species have become extinct, so the very few collected pupae (less than half-a-dozen for rare species) are irreplaceable scientific assets. I therefore felt obliged to take the opportunity to examine also the conspicuous granules that adorn the dorsal surface of blepharicerid pupae, especially because there are significant differences between the two subgenera. The function of these tiny integumental outgrowths is obscure, but the discussion draws attention to analogous structures of other aquatic insects and refers to a correlation between the orientation of the granules and flow patterns around blepharicerid pupae. The shape and fine structure of the gills are discussed in comparison with *Edwardsina* pupae.

MATERIALS AND METHODS

Pupae were sourced from the collection of B. R. Stuckenberg (Natal Museum, Pietermaritzburg, South Africa). They had been fixed in 70–85 % ethanol. Stuckenberg found them at the following places on the given dates:

P. umbra, *P. pamela* and *P. rivalis*: Perinet, east-central Madagascar, ca. 960 m, mid-December 1955. *P. ingens*, *P. silva* and *P. alexanderi*: Antsomangana River, Manjakatombo Forest Station, Ankaratra Massif, central Madagascar, 4–10 January 1956.

As far as possible, the gills were cleaned using a thin jet of ethanol produced by a syringe. Ultrasound was not used because of the high risk of artefact production. Cross-sections of the gills were made by using thin razor blades. Pointed pieces of adhesive film (Scotch Pressure Sensitive Tape) proved appropriate for detaching tissue from the tiny (dried) atria. When contamination was removed from granules by light maceration with KOH, artefacts were excluded by previously checking untreated structures. The preparations were dehydrated (mixture of ethanol and acetone with 10, 30, 50, 70, 90 and finally 100 % acetone), dried (critical point apparatus model 020, Balzers), coated with gold (Sputter Coater S150B, Edwards), and examined with a scanning electron microscope (Cambridge S90-SEM).

RESULTS

Blepharicerid pupae are found attached to rock surfaces almost exclusively in fast-flowing clean water, with the head pointing downstream. They obtain their oxygen by means of conspicuous spiracular gills arising from the prothorax (Fig. 3). In the subfamily Edwardsiniinae, these respiratory organs are composed of a transverse, oval baseplate in the region of the prothoracic spiracle, and, at least in species primitive in this respect, a number of longitudinal lamellae (often 9) protruding vertically from this plate (Stuckenberg 1958; Zwick 1977 1981a,b; Arens 1995 1998). The baseplate and all erect gill processes are covered with a plastron, the air film of which is directly connected via a slit-like spiracular opening with the atrium of the tracheal

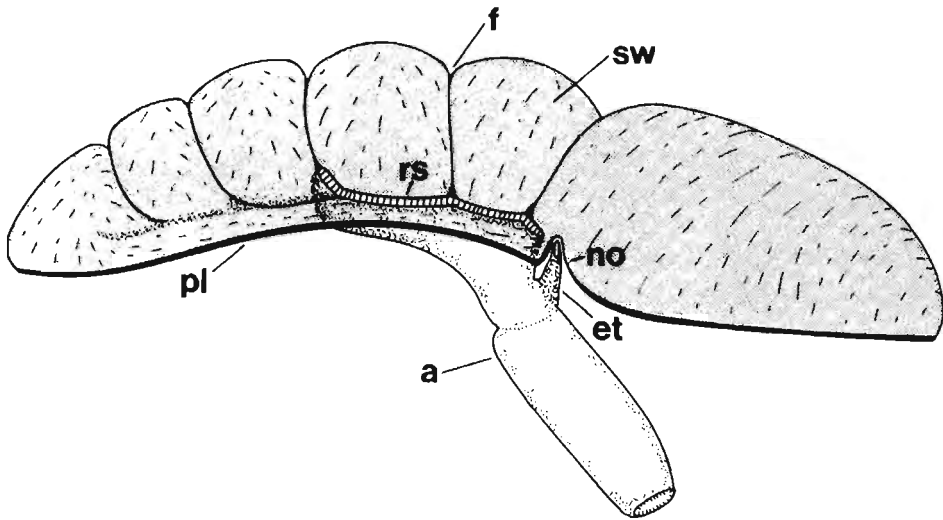


Fig. 1. Structural details of pupal spiracular gills in *Paulianina* (*Eupaulianina*) pupae. The gills consist of a baseplate (pl), swellings (sw) or erect lamellae, an atrium (a), fissures (f) between them, a respiratory suture (rs), posterior notch (no) and ecdysial tube (et); right organ.

system underneath (Fig. 1). This so-called respiratory suture extends in a variable course across the baseplate, but starts near a usually well-defined notch at the posterior margin of the gill, where the ecdysial tube ends outside the baseplate in each species of Edwardsiniinae (Figs 1, 5, 67). The ecdysial tube, which lacks the

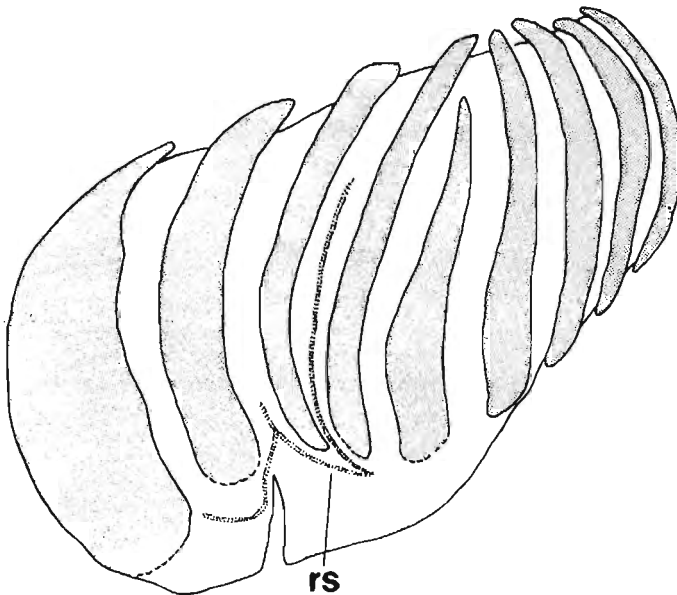


Fig. 2. Schematic drawing of the left spiracular gill of *Paulianina ingens*, showing the complex course of the respiratory suture (rs).

spongy chitinous filling of the respiratory branch of the atrium, is needed only for the drawing-out of the larval trachea; it collapses after larval-pupal ecdysis, so that its external opening, the ecdysial pore, is closed after moulting (Weber 1933; Keilin 1944; Hinton 1947; Hennig 1950).

Within the genus *Paulianina*, this gill structure is found in a rather primitive version in the subgenus *Paulianina* s.s., whereas the species of the subgenus *Eupaulianina* have respiratory organs that are unique among Edwardsininae in some aspects, even if they still correspond to the ground plan of the subfamily. Therefore, I will first present the findings on *Paulianina* s.s.. The information regarding the pupal shape and the gill characters discernible by light microscopy are partly a synopsis of the detailed descriptions given by Stuckenberg (1958). He also previously recorded the variable density of the granules that decorate the dorsal surface of *Paulianina* pupae, except for the pterothecae and the thecae of the halteres (Figs 5, 25). Therefore, my comments on these structures can be restricted to their shape and pattern of orientation.

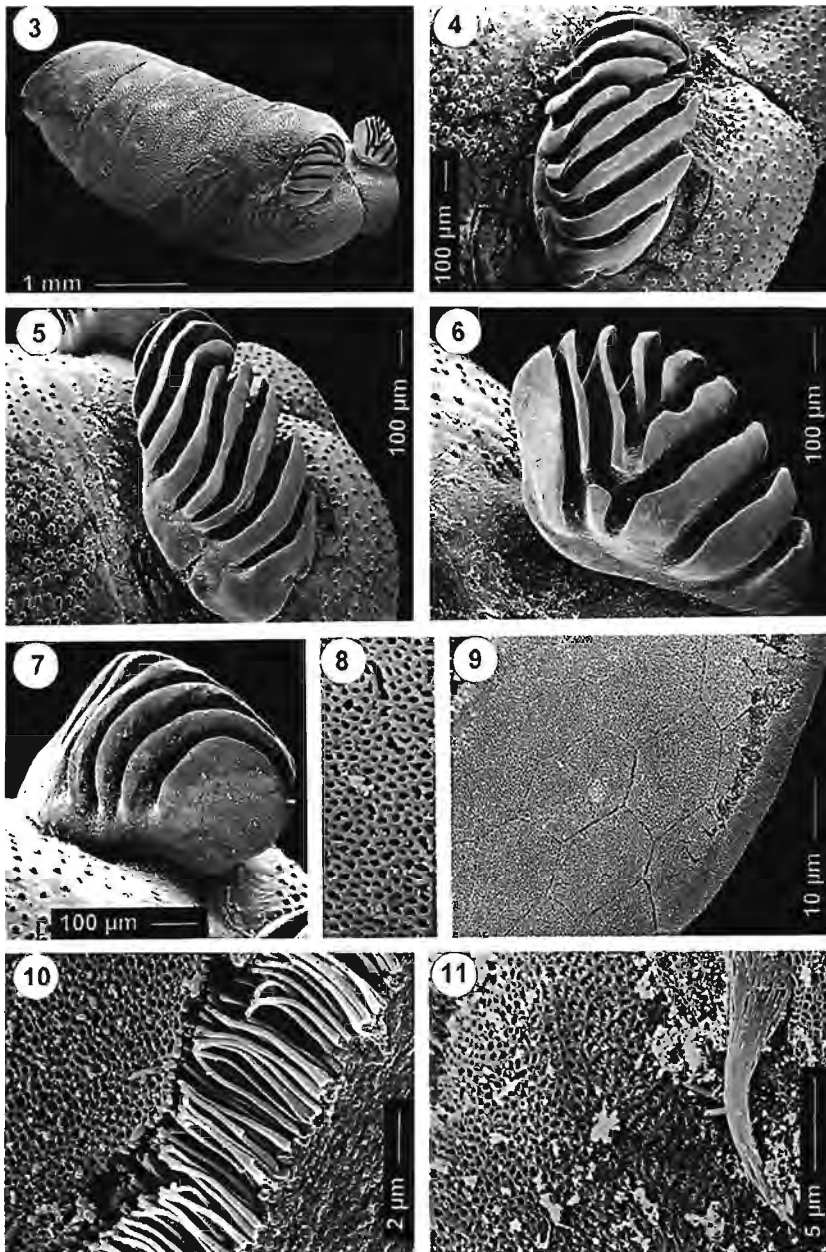
Subgenus *Paulianina* s.s.

The pupae of this subgenus are strongly vaulted compared with the species of *Eupaulianina*, and their prothorax descends steeply at the apex (Fig. 3). The marginal part of the prothorax is curved underneath the pupa and its anterior portion may even face downwards. The surface of the gills is differentiated into 9 erect protuberances that have the form of solid lamellae or hollow transverse bulges. The granules on the dorsal surface of the pupae carry a group of spines near their terminal end or they have the shape of humps with no sculptural markers. The orientation of the granules is therefore discernible only on some parts of integument.

Paulianina (*P.*) *ingens* Stuckenberg, 1958

Length of pupae: 6.00 mm; 6.62 mm (Stuckenberg 1958). Examined specimen: 6.1 mm.

The gills of *P. ingens* are composed of an oval baseplate crowned by a set of 9 solid erect lamellae (Figs 3–7). Compared with other Edwardsininae, the respiratory organs are relatively small. They are placed on the slightly vaulted dorsal region of the prothorax and insert high above the lateral margin of the thorax, separated from it by an almost 700 µm wide and steeply descending space (Fig. 3). Medially, the gills are separated from each other by a gap of approximately 350 µm in width. The 9 lamellae completely cover the baseplate, except for a narrow anterior strip (Fig. 6) and a bare region along the posterior margin on the outer half of the gill (Fig. 5). A deep notch, opposite to the space between lamellae 2 and 3 (counted from the outside), extends longitudinally across this bare region and almost divides it into 2 parts (Figs 2, 5). The closed external opening of the ecdysial tube lies in the ground of the notch, near the posterior margin of the baseplate.



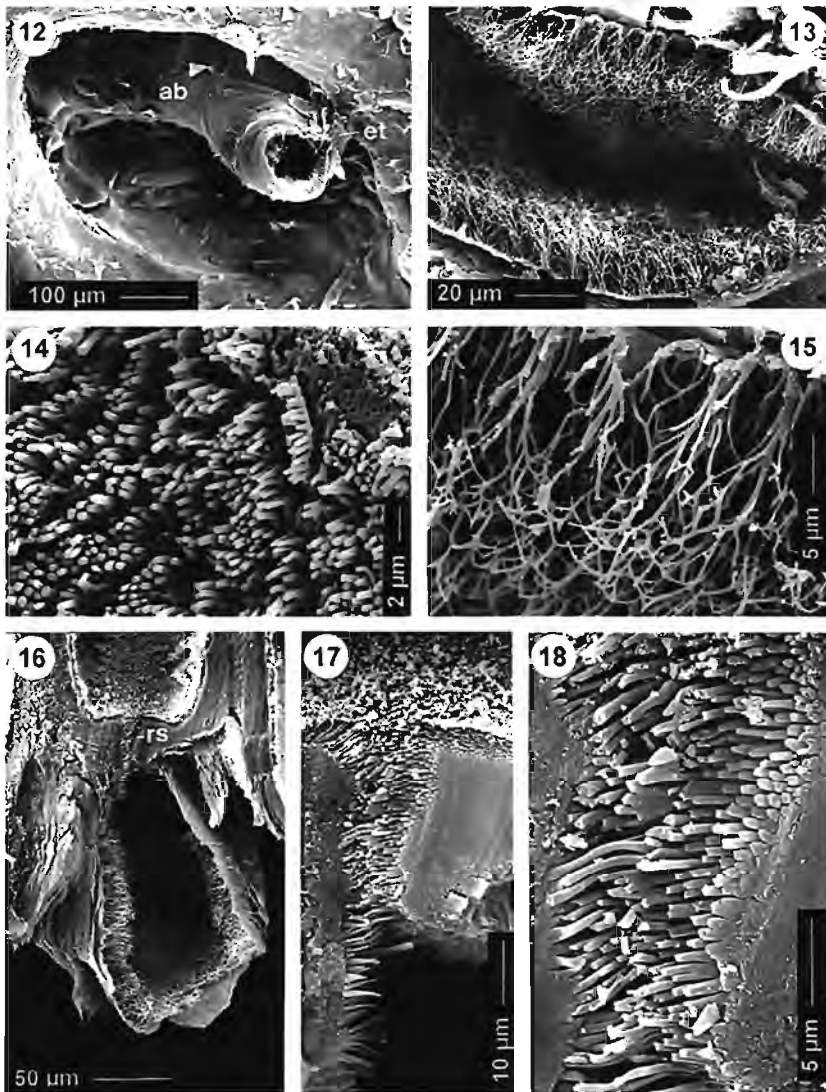
Figs 3–11. *Paulianina* (*P.*) *ingens*, external gill structures. 3. Pupa with its conspicuous respiratory organs on the prothorax. 4. Right gill, latero-frontal view. 5. Rear side of the right gill; note the deep indentation of the posterior margin. 6. Left gill, frontal view. 7. Medial face of the inner lamellae of the left gill. 8. Typical plastron surface; example taken from the baseplate near the notch. 9. Plastron surface of the uppermost region of lamella 4, with its distinct hexagonal tessellation (and some damage); width of the figure: 4.5 μm . 10. Section through the plastron near the ground of an interlamellar space. 11. Irregular plastron structure above the respiratory suture in the region lateral to the notch.

On the posterior part of the gills, all lamellae have an almost longitudinal orientation and run parallel to each other (Fig. 5). However, the 4 outer lamellae, which are convexly vaulted (seen from the outside), curve inwards at the anterior end, whereas the 5 inner lamellae have the opposite shape (Figs 4, 6). In spite of this frontal convergence of the 2 groups of lamellae, a similar distance between all lamellae is obtained by the shortening of lamellae 4–7 in the middle region of the gill (Figs 4, 6). Additionally, these lamellae tend to fuse in pairs with their anterior ends (4 with 7, 5 with 6), although this fusion is always confined to the lower part of the respiratory processes (Fig. 6). At least in the examined specimen, lamella 8 ends independently from its counterpart (lamella 3) near the anterior margin of the gill, but is deeply indented opposite to the end of the adjacent lamella 7. An analogous concavity is present on the left lamella 4, opposite the end of lamella 5 (Fig. 6).

The fine structure of the plastron is similar on all parts of the gill, except for the respiratory suture. The plastron consists of a dense carpet of trichomes and a perforated membrane lying on the carpet and associated with the end of the trichomes ('plastron with cover membrane'; Arens 1995, Figs 8, 10). The density and diameter of the aeropyles decreases from the baseplate towards the upper region of the respiratory processes; this is a typical modification in Edwardsininae pupae with this type of plastron (Arens 1995 1998). A hexagonal tessellation of the plastron surface is clearly defined on the upper portion of some lamellae (Fig. 9), but is absent or indistinct on other parts of the gill.

A modified structure of the plastron, characterised by irregular apical connections of the trichomes, unusually wide aeropyles and other peculiarities (Fig. 11), is found on the respiratory suture, which has a more complicated course than that described by Stuckenberg (1958). The suture consists of a transverse sinuous section that runs directly behind lamellae 2–4 across the bare region of the baseplate, and 2 longitudinal branches extending into the 2nd and 3rd interlamellar spaces (Fig. 2). The transverse section starts at the outer posterior end of lamella 2, curves around the foot of this lamella and reaches, after a short diagonal run, the beginning of the 2nd interlamellar space. Here, a branch forks into this space, whereas the main part of the suture continues at a sharp angle and runs with a curved course backwards to the region posterior to the 4th interlamellar space. At the end of this transverse section of the suture, a second branch arises at a hairpin-like angle and extends far into the 3rd interlamellar space. All parts of the suture are readily discernible as a translucent line by light microscopy, and as a strip with an irregular plastron structure by electron microscopy. The branch on the floor of the 2nd interlamellar space is short, despite a much longer strip with irregular plastron structure being found here.

The atrium and the respiratory suture resemble those of *P. umbra* in structure (see Arens 1995). The atrium follows the general course of the respiratory suture on the underside of the gill (from the lateral side of the ecdysial pore up to the middle of the 3rd interlamellar space), but the angles of the suture and its short branch into the 2nd interlamellar space are not accordingly reflected by atrial bulges (Fig. 12). The respiratory suture is protected against entry of water by the plastron outside, and by numerous tightly packed crossbars linking the walls of the slit, which is 25–30 μm deep (Figs 16–18, 14). Underneath the integument, the respiratory suture widens to the felt-lined chamber of the atrium (Figs 16, 19), the walls of which are coated with



Figs 12–18. *Paulianina (P.) ingens*, structure of the atrium. 12. View of the undersurface of the left gill, showing the rear side of the upper half of the atrium; ventral half removed (et = ecdysial tube, ab = atrial branch underneath 4th interlamellar space). 13. View into the vault of the (opened) horizontal part of the atrium. 14. Plastron structures on the wall of the opened respiratory suture near the fork into the 2nd interlamellar space; the transition to the plastron type of the external gill structure is seen in the right upper corner. 15. Wall structures of the horizontal section of the atrium. 16. Section through the respiratory suture (rs) and the atrial branch underneath the 3rd interlamellar space; because the atrium is obliquely cut, its lumen appears wider than it is *in vivo*. 17. The extremely narrow respiratory suture between the plastron outside and the atrium inside. 18. Fine structures of the respiratory suture.

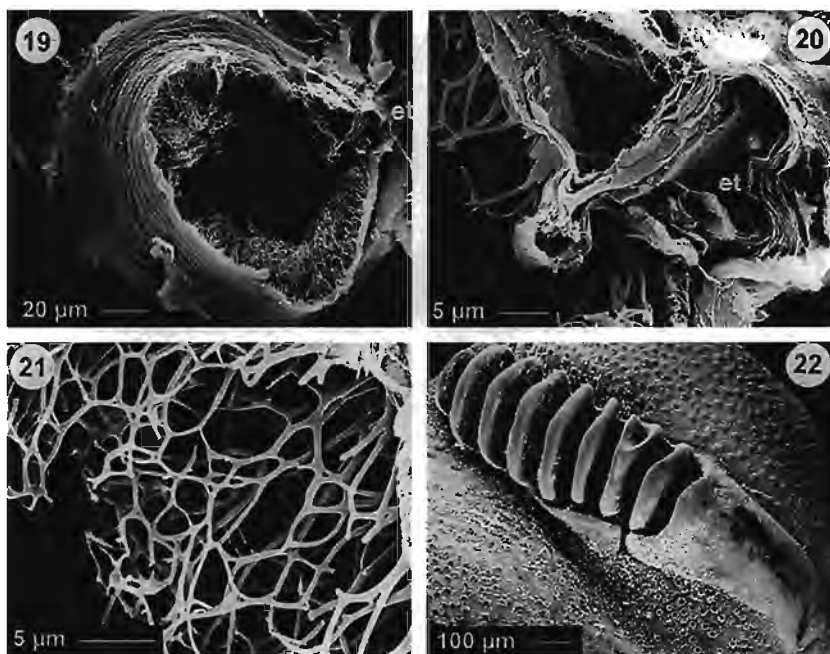
a cuticular network composed of filiform branched trichomes (Figs 13, 15, 19, 21). With regard to the structural details of the atrium, there are only slight differences

compared with those of *E. umbra*. In both species, the felt never completely fills the atrium, the cuticular network of the felt-lined chamber is modified gradually near the respiratory suture into a carpet of smooth trichomes (Fig. 17), and the ecdysial tube reaches downwards to half the height of the atrium. In *E. ingens*, however, the threads of the cuticular network (Figs 15, 21) are thinner than those in *E. umbra*, and the central lumen of its atrium is comparatively wider (Figs 16, 19).

The granules on the tergites of the pupa have the shape of round humps with a diameter of 15–25 μm (Figs 87–89). Their surface is smooth (Fig. 89) or, in most thoracic granules, wrinkled (Fig. 88) or rough, because they are studded with tiny tubercles (Fig. 87). Granules on the abdomen (except on the first tergite) carry a transverse row or a small group of short spines directed caudally in most cases (Fig. 89). The orientation of the thoracic granules is obscure because spines are missing here, and an asymmetry is not indicated by the arrangement of the other sculptural decorations.

Paulianina (P.) umbra Stuckenberg, 1958

The gills of this species were examined in the first part of my series (Arens 1995). The baseplate is differentiated into 8 lamellae and 1 transverse bulge (Fig. 22). Most regions of the bulge are covered by a 'hair-carpet plastron' (composed of isolated trichomes), whereas a plastron with cover membrane is found on all other gill surfaces. The respiratory suture is short and extends as a sigmoid line from the posterior notch onto the 3rd interlamellar space.



Figs 19–22. *Paulianina (P.)* species. 19–21. *P. ingens*, structure of the atrium. 19. Section through the atrium near the internal opening of the ecdysial tube (et); compare with Fig. 13. 20–21. Details: section through the collapsed ecdysial tube (et), and wall structures of the atrium. 22. *P. umbra*, right gill.

The granules resemble those of *P. ingens* in shape and size, but their base is circumferentially adorned by a variable number of thorn-like protuberances (Fig. 90). As far as is discernible on the fragments of the single exuvia at my disposal, abdominal and thoracic granules do not differ significantly from one another, although modifications are found on some regions of the pupa, especially on the lateral and terminal surfaces of the abdomen and on areas near the intersegmental sutures. Here, the thorn-like protuberances are often modified into parallel wrinkles (Fig. 91), or are reduced in number or size (Fig. 92). If only a few thorns remain on the abdominal granules, they are usually located on their posterior face.

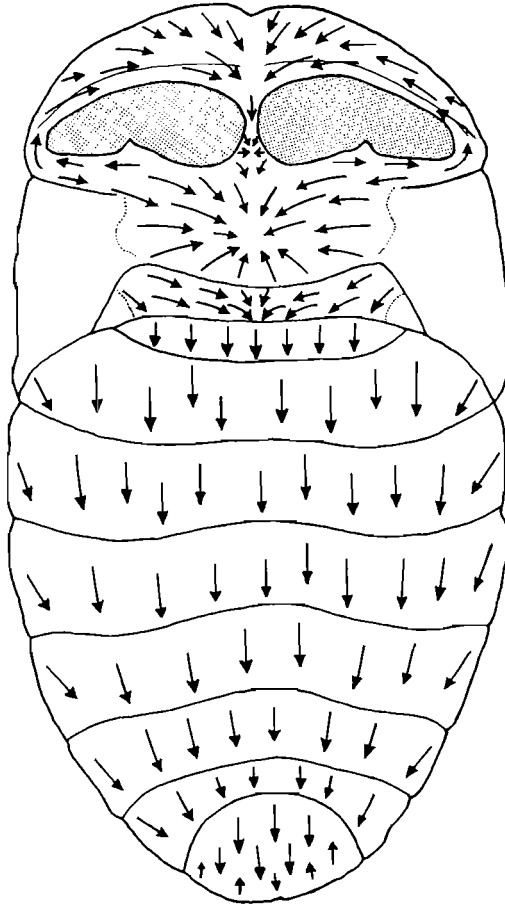


Fig. 23. Pattern of orientation of the granules on the dorsal surface of *Eupaulianina* pupae. The tip of the arrows indicates the principal position of the small terminal blister on the granules in this region.

Subgenus *Eupaulianina*

With the exception of the less flattened *P. pamela*, the pupae of this subgenus are strongly compressed dorsoventrally (Figs 40, 41) and therefore differ in shape from

other Edwardsininae; they resemble the pupae of *Philorus* (subfamily Blepharicerinae). The portion of the prothorax anterior to the respiratory organs is relatively broad, descends slightly and is marked by 2 transverse ridges (Fig. 24). The lateral margins of the thorax are sharp, and only a narrow anterior strip of the prothorax is curled underneath the body. Instead of erect lamellar protrusions, which are found only in 1 species (*P. species I*; Stuckenberg 1958), 5 or 6 gentle swellings or broad elevated areas are present on the gills.

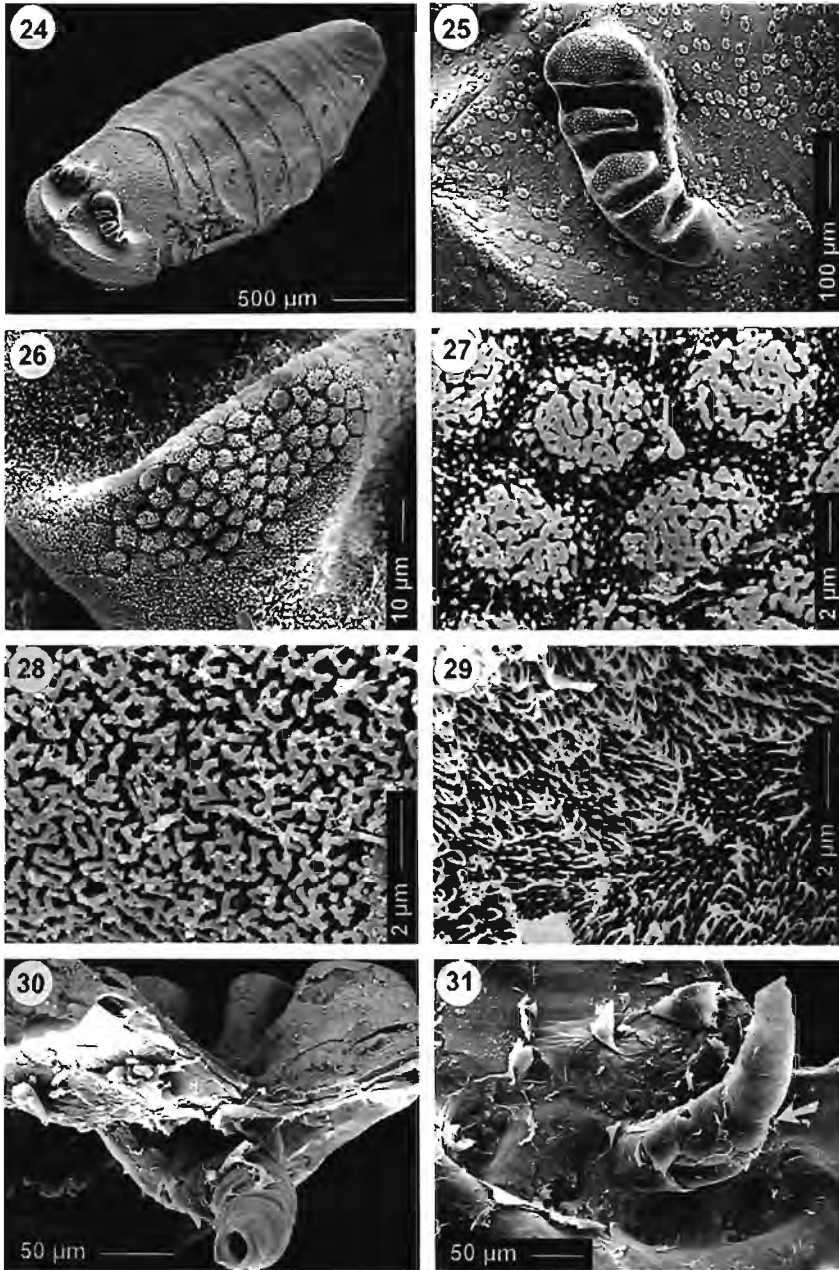
The granules on the dorsal surface of the *Eupaulianina* pupae possess a small, hollow, terminal blister in almost every case (Figs 93–103). Most of them are additionally adorned with a group of spines, and occasionally the centre is marked by a pore. The orientation of the granules is similar in all species and has the pattern shown in Fig. 23. The granules on the abdominal tergites are usually oriented caudally; this means that their terminal blister is directed towards the end of the pupa. On the marginal area of the tergites, the arrangement of the granules often changes to a caudo-medial orientation, and some granules with blisters pointing forwards may occur on the last tergite. The granules of the thorax show a more complex pattern of arrangement. Those of the metanotum have an orientation that can be symbolised by lines converging from the sides and the anterior region to the middle of the posterior margin of the tergite. The small thecae of the halteres are not granulated. The granules of the mesonotum are oriented, by and large, from all sides to a point near the middle of the tergite. The pterothecae, which form the lateral part of the mesothorax and embrace the metanotum and the first 2 abdominal tergites, always have a smooth surface.

The orientation of the granules on the prothorax varies in a manner such that a whorl-like pattern around the gills results. On the narrow strips of prothoracic integument posterior to the gills, the terminal blister of most granules points outwards or obliquely forwards, so that their orientation is strikingly different from the adjacent mesothoracic outgrowths. On the portion of the prothorax anterior to the respiratory organs, the granules are oriented along lines that converge from both sides to the space between the gills; granules directly in front of the gills usually have blisters pointing inwards, whereas most granules on the anterior region of the prothorax have a caudal or medio-caudal orientation. This applies also to the downturned apex of the prothorax on which the blisters therefore are directed forwards or upwards. The space between the gills is only sparsely granulated. Here, most blisters are directed caudally or medially, so that a diametrically opposite orientation of granules may result along the middle line of the pronotum, in particular on its posterior region.

P. (E.) pamela Stuckenberg, 1958

Length of pupae: 2.75 mm; 3.15 mm (Stuckenberg 1958). Examined specimen: 3.3 mm.

The pupae of this species are more elongated and less flattened than other species of the subgenus (Fig. 24). The pear-shaped gills (Fig. 25) are very small and insert on the concave middle region of the thorax, some distance from its anterior and lateral margins. The broad prothoracic portion anterior to the respiratory organs is marked



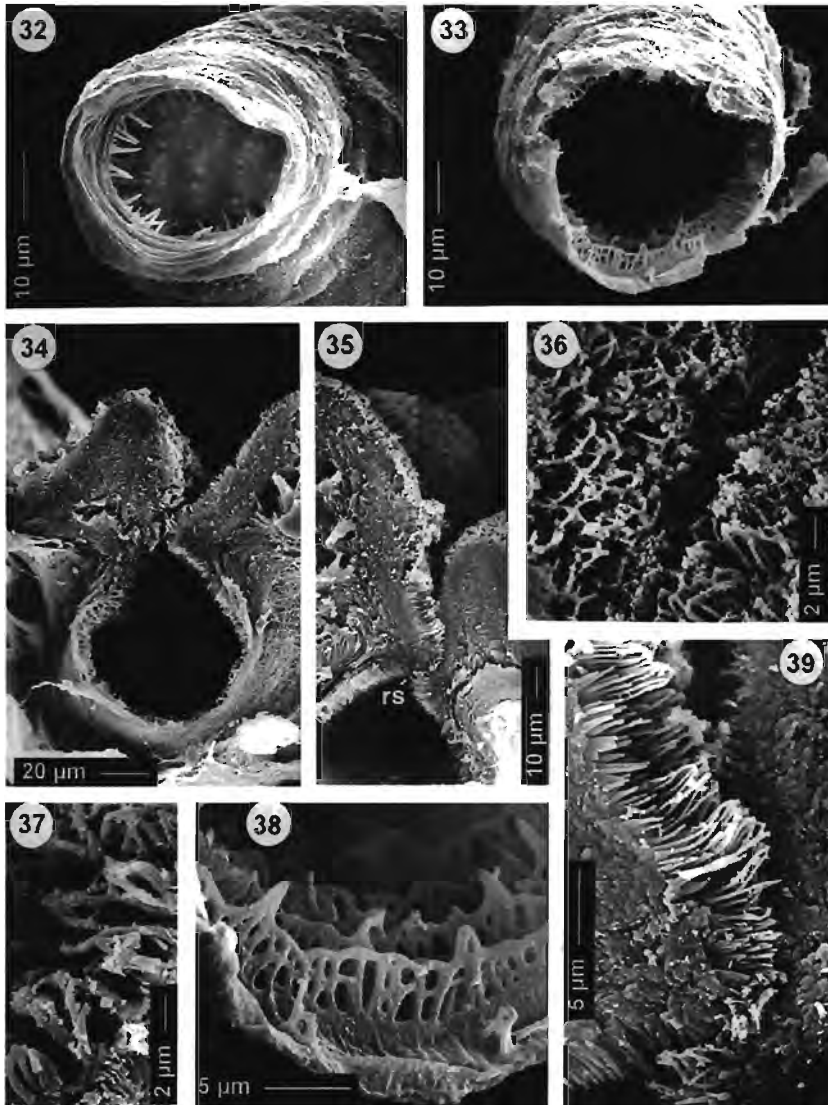
Figs 24–31. *Paulianina* (*E.*) *pamela*. 24–25. Pupa and left gill. 26. Tessellated plastron surface on the uppermost region of the 2nd respiratory elevation. 27. Fine structure of the tessellated region of the plastron. 28. Plastron structure on the lower levels of the respiratory elevations. 29. Plastron structure in the bases of the valleys between the elevations. 30. Section through the integument behind the right gill, showing the respiratory elevations, the twisted atrium and the short ecdysial tube. 31. Frontal view of the atrium; note the narrowing (arrow) between the slender ventral and the wider (slightly damaged) dorsal part.

by a prominent transverse ridge. The vaulted surface of the gills is fissured: 5 rounded longitudinal elevations are separated by 4 relatively wide trenches (Figs 25, 26). The innermost elevation is the largest and is posteriorly elongated into a gradually descending rim that extends transversely along the posterior margin of the gill, up to the notch at which the ecdysial tube ends. This notch is situated next to the end of the 2nd elevation but is indistinct. The respiratory suture is short and runs almost directly from the posterior notch onto the middle of the 3rd trench (schematic drawing in Arens 1995). In dorsal views of the gill (also by light microscopy), the suture seems to be comparatively wide (Fig. 36), but this impression results because the respiratory suture opens at the base of a valley-like depression of the gill integument.

The structure of the plastron varies considerably. The floor of the trenches is covered by a carpet of trichomes, which are apically fused with another only in a few cases and then form arches or multiple arches (Fig. 29). The plastron next to the respiratory suture is not significantly modified (Fig. 36). The carpet of trichomes extends from both sides into the integumental depression above the suture (Fig. 35). Towards the higher levels of the gill, the trend towards apical fusion of the trichomes increases, so that the hair-carpet plastron is modified on the flanks of the elevations and on the marginal areas of the gill, into a plastron type that is characterised by numerous irregular horizontal anastomoses between the apices of vertical trichomes (Fig. 28). A similar plastron structure is also found on the uppermost region of the elevations and on the transverse posterior rim. Here, however, the trichomes lying on small, *ca.* 5 μm long, polygonal (often pentagonal) areas are connected with each other by a dense layer of irregular anastomoses, so that a striking tessellation of the gill surface results (Figs 26, 27). Neighbouring polygons are separated by narrow strips of short individual trichomes (Fig. 27).

The atrium is a slightly bent tube with a length of *ca.* 300 μm and a diameter of only 50–80 μm . Its twisted shape, caused by the sigmoid course of the respiratory suture, is most readily visible in rear views of the organ (Fig. 30). A circular indentation subdivides the tube into a slender ventral and a wider upper half (Fig. 31). An analogous fold is also found ventral to the internal ecdysial pore in other *Edwardsininae* pupae, but has hitherto not been adequately noticed by me (Arens 1995 1998). Because of the oblique orientation of the atrium underneath the prothoracic integument, the ecdysial tube is short (*ca.* 40 μm), although it reaches from the external ecdysial pore downwards to the narrowed middle region of the atrium (Fig. 30).

A view through the end of the trachea into the ventral opening of atrium shows that the spongy chitinous material which usually forms a conspicuous coat on the inner walls of the atrium in *Edwardsininae* pupae, is only meagrely developed in *P. pamela* (Fig. 32). Lash-like trichomes hem the border between the trachea and atrium, and faint signs of further circles of wall structures are visible inside the tube. Cross-sections demonstrate that these circles consist of low rings formed by thicker outgrowths and irregular transverse anastomoses between them (Fig. 38). A similarly skimpy, only 4–5 μm high, coat is also found on the walls of the horizontal section of the atrium underneath the respiratory suture (Figs 34, 37). The suture itself, however, is blocked in similar manner to that in other *Paulianina* species, by an extremely dense filling of crossbars (Figs 34, 35, 39).



Figs 32–39. *Paulianina* (*E.*) *pamela*, structure of the atrium. 32. View through the end of the trachea into the lowest part of the atrium. 33. Section through the atrium ventral to the level of the internal opening of the ecdysial tube. 34. Section through the respiratory suture (artificially widened) and the horizontal part of the atrium. 35. The respiratory suture (rs) between the plastron outside and atrium inside. 36. Plastron surface above the respiratory suture; some trichomes are encrusted by debris. 37. Wall structures of the horizontal section of the atrium (detail of Fig. 34). 38. Wall structures of the ventral part of the atrium (detail of Fig. 33). 39. Fine structures of the respiratory suture (detail of Fig. 35).

The widely separated, cupola-shaped granules are marked by uneven or wrinkled edges and a large terminal blister, which may have half the size of the oval main part (Figs 93–95). Contrary to the observations of Stuckenberg (1958), the size of the

granules (*ca.* 10 μm) varies only slightly in the examined specimen, but those on the abdomen have smaller blisters and carry, in contrast to the thoracic granules (Figs 93, 94), additionally a set of small spines (Fig. 95). A relatively large number of granules deviates with respect to the orientation from the typical pattern outlined above (Fig. 23), especially on the thorax and the terminal tergite of the abdomen.

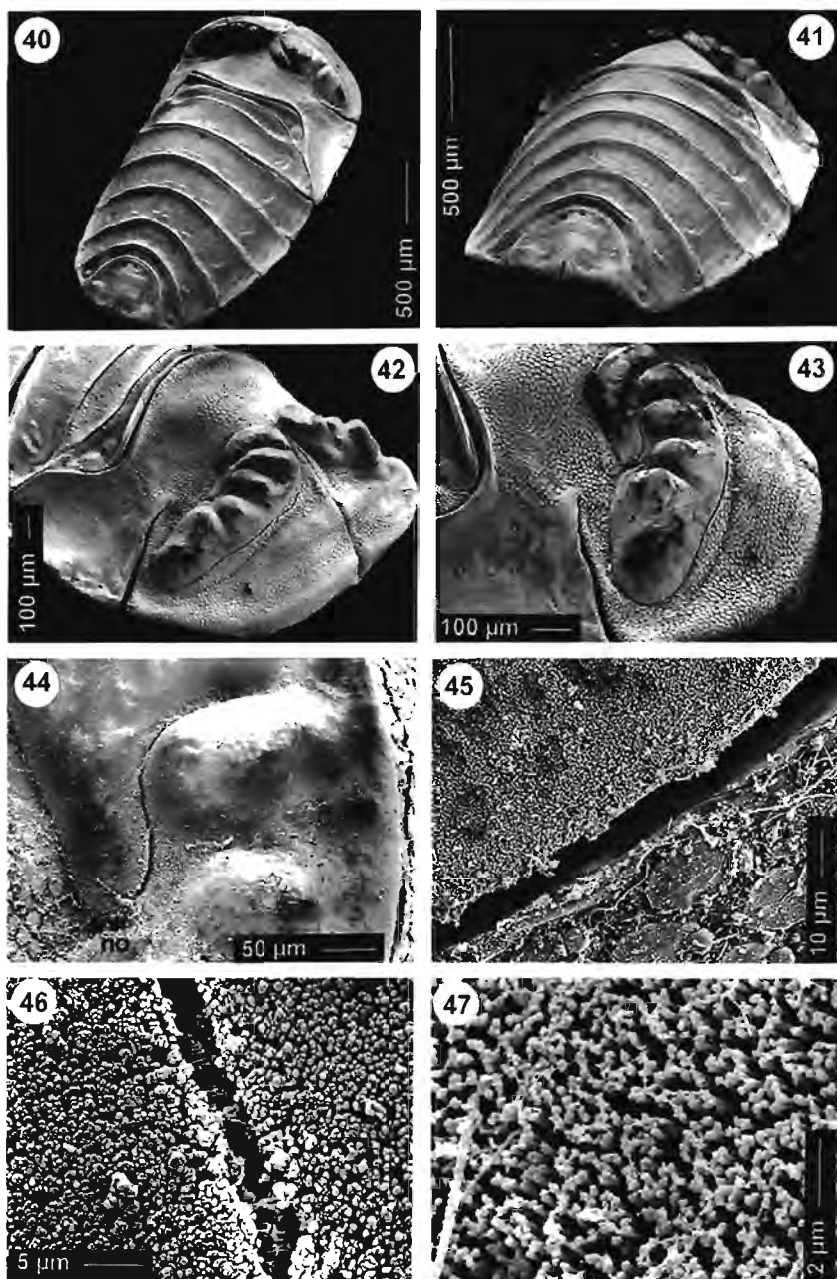
P. (E.) rivalis Stuckenberg, 1958

Length of pupae: 3.63 mm; 3.75 mm (Stuckenberg 1958). Examined specimen: 3.5 mm.

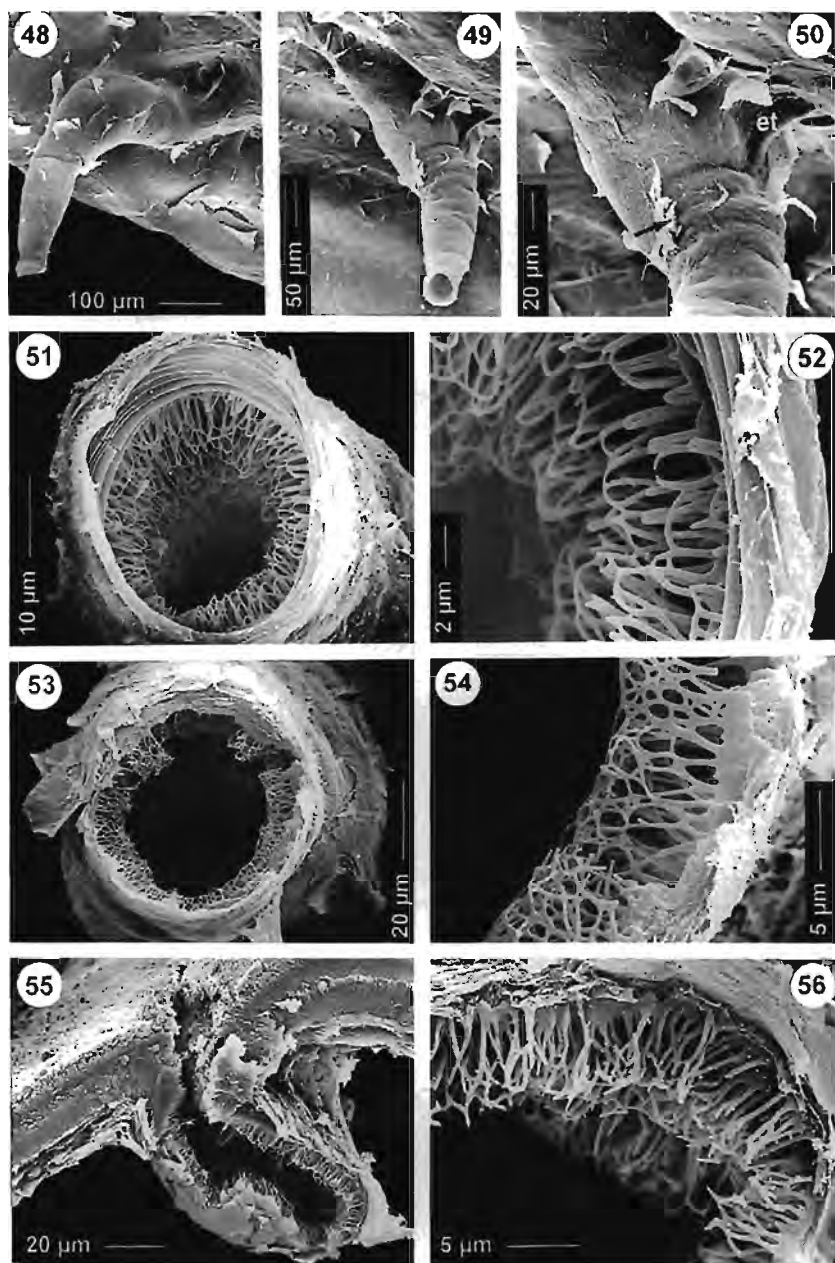
The strongly compressed, shield-like pupae of *P. rivalis* possess oval spiracular gills extending transversely from the middle line of the prothorax almost down to its lateral margin (Figs 40–43). The respiratory organs have a relatively even upper relief, which is formed by 5 gently rounded elevations separated from each other by shallow valleys (Figs 42, 43). The outermost elevation is by far the largest and completely occupies the lateral third of the gill. The 3 intervening elevations resemble each other in shape and size. They are shortened posteriorly, and are here embraced by the innermost elevation, which forms the bent apex of the gill and reaches with its tapering end onto a flat plain-like region behind the 3 middle elevations (Fig. 43). This flat gill area is separated from the hilly anterior part by the respiratory suture that runs as a distinct sinuous line from the ecdysial pore into the 3rd valley (Figs 43, 44). The position of the ecdysial pore is marked by a recess in the kink of the posterior gill margin.

All surfaces of the gill are covered with a hair-carpet plastron, the trichomes of which are densely packed on the elevated regions of the gill, but are less numerous at the base of the valleys and on the flat posterior region (Figs 46, 47). On the top of the elevations, a surface pattern similar to that of *P. pamela* is found, but this results only from a particularly dense arrangement of trichomes and not from horizontal anastomoses between their apices (*cf.* Fig. 59). Apical fusion of trichomes, however, occasionally occurs inside the plastron of the valleys (*cf.* Fig. 60). Above the respiratory suture, the dense carpet of vertical trichomes is interrupted so that horizontal struts which block the respiratory suture against the entry of water are visible (Fig. 46). Fixation or dehydration of the pupae obviously causes artificial widening of some sections of the suture, but I assume that Fig. 46 approximates a natural state. A conspicuous cleft under the marginal region of the gill (Fig. 45) may also be artefactual. It establishes, nevertheless, the clear demarcation of the gill from the prothoracic integument.

The *ca.* 400 μm long atrium (Figs 48, 49) resembles in shape that of *P. pamela*, but projects more perpendicularly from the underside of the gill so that the short ecdysial tube is readily visible at the upper posterior face of the organ (Fig. 50). Again, a circular indentation is present on the atrial wall next to the internal opening of the ecdysial tube; it forms a border between the ventral uneven section of the atrium and the wider upper part with its smooth surface (Figs 49, 50). The internal wall structures of the atrium are more developed, as they are in *P. pamela*. Near the lower end of the atrium, the walls are densely carpeted by smooth, hair-like trichomes so that there is a strong contrast in appearance relative to the adjacent trachea (Figs 51,



Figs 40–47. *Paulianina* (*E.*) *rivalis*. 40–41. Pupa, oblique dorsal view and profile in rear view. 42. Thorax of the pupa; latero-frontal view. 43. Right gill; lateral view. 44. The sigmoid respiratory suture between the posterior notch (no) and the 3rd valley. 45. Gap between the specialised gill integument and the prothoracic tergite. 46. View into the respiratory suture; tips of the plastron trichomes with artificial caps caused by the extremely prolonged coating with gold. 47. Typical plastron surface; example taken from the region near the posterior notch.



Figs 48–56. *Paulianina (E.) rivalis*. 48–49. Atrium on the undersurface of the left gill; frontal and rear side. 50. Border between the dorsal and ventral part of the atrium (arrow), and the ecdysial tube (et). 51. View through the end of the trachea into the lowest part of the atrium. 52. Detail: wall structures. 53. Section through the middle region of the ventral part of the atrium. 54. Detail: wall structures. 55. Section through the respiratory suture (artificially widened) and the horizontal part of the atrium. 56. Detail: wall structures.

52). In higher regions of the atrium (Figs 53, 55), numerous branched trichomes form a spongy (ca. 5–10 μm high) coat on the inside walls (Figs 54, 56), although even here, the atrial lumen is little constricted by the outgrowths. A small area around the internal ecdysial pore is free of trichomes. The respiratory suture is obstructed, as usual, by numerous crossbars so that only a narrow system of interstices remains open. Analogous to *P. pamela*, the respiratory suture lies in the ground of a valley-like depression of the gill integument, into which the plastron extends from both sides. The course of the suture is therefore very distinct in dorsal views of the gill (Fig. 43), unlike in species with a suture concealed by an evenly horizontal plastron (e.g. *P. ingens*).

The 12–15 μm long granules on the dorsal surfaces of the pupae are tightly packed and coin-shaped, with a slightly concave dorsal face and smooth edges (Figs 96, 97). A small blister is inserted in a terminal indentation. Additionally, there is a transverse row consisting of 1–5 spines on most abdominal granules.

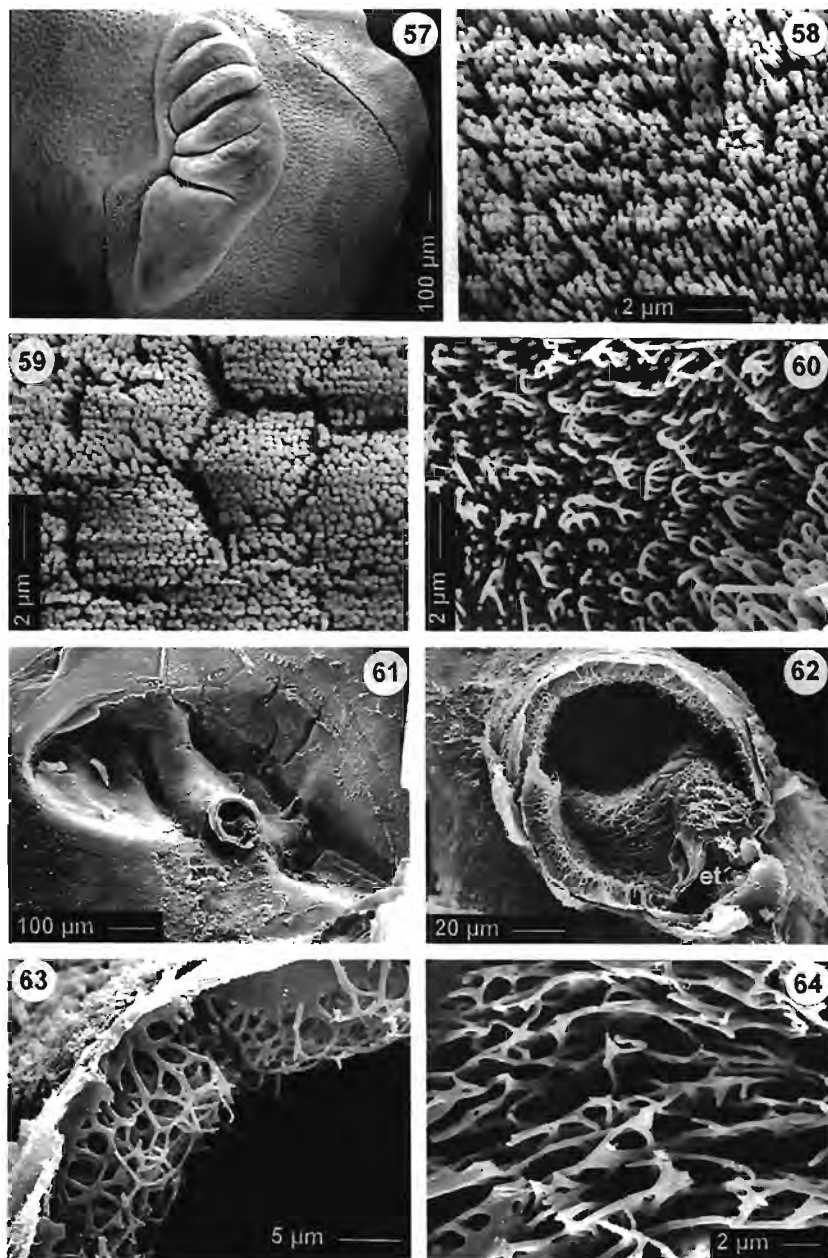
P. (E.) silva Stuckenberg, 1958

Length of pupae: 4.00 mm; 5.15 mm (Stuckenberg 1958). Examined specimen: 5.1 mm.

Pupae of *P. silva* have the same strongly flattened body form as those of *P. rivalis* and *P. alexanderi*. The gills are kidney-shaped (Fig. 57), with a medially angled anterior margin, and resemble in many aspects those of *P. rivalis*. However, there are 6 swollen elevations instead of 5, and narrow furrows take the place of the shallow valleys between them. The outermost elevation is again the largest, whereas the 5 inner swellings are similar to one another with respect to size and elongated shape, except elevation 2, which widens out anteriorly and covers the angled middle portion of the respiratory organ. Analogous to *P. rivalis*, a strip of flat gill integument is found posterior to the series of elevations (Figs 1, 57). It is not clearly separated from the 2 innermost swellings and extends outwards up to the base of the large elevation 1 and embraces the ecdysial pore, which is situated directly posterior to the outermost furrow. In another examined specimen (see also Stuckenberg 1958), the strip is a little shorter so that the ecdysial tube is not enclosed by gill integument.

The plastron structure (Figs 58–60) is similar to that of *P. rivalis*.

Some comments are necessary regarding the course of the respiratory suture in *P. silva*. Stuckenberg (1958) believed that the suture is branched in this species and, indeed, bright light lines are visible under intense illumination, on the floor of all furrows of the gill, a feature that may have caused the erroneous interpretation. However, these bright lines result from the horizontal gill integument being more translucent at the base of the furrows (in dorsal views of the gill) than on the steep flanks of the elevations. The actual course of the respiratory suture is simple and similar to that of *P. umbra*, *P. pamela*, and *P. rivalis*. The suture runs from the ecdysial pore along the end of the 2 middle elevations and terminates, after a short bend, at the beginning of the 3rd furrow, only ca. 50 μm beyond the fork of furrows at the base of elevation 4 (Figs 1, 57). Except for this very short section, the suture never sends branches into the furrows; moreover, the trench posterior to elevation 4 must clearly be distinguished in this regard from the 'real' respiratory suture posterior to elevations 2 and 3.



Figs 57–64. *Paulianina* (*E.*) *silva*. 57. Right gill; fronto-lateral view. 58. Plastron surface on the dorsal region of the innermost elevation. 59. Plastron surface on the same elevation but near the furrow. 60. Plastron surface at the base of the fissure between 4th and 5th elevations. 61. View of the undersurface of the left gill, showing the uppermost part of the atrium and the conspicuous bulge underneath the fissure between the 3rd and 4th respiratory elevations. 62. Detail: section through the atrium at the level of the internal opening of the ecdysial tube (et). 63–64. Wall structures of the atrium in this region; cross section and dorsal view.

The view of the underside of the gills also leads initially to a misinterpretation (Fig. 61). The atrium seems to extend as a rounded bulge completely over the bottom of the 3rd furrow of the gill. However, cross-sections show that this bulge results exclusively from the enormous depth of this furrow and is therefore not part of the tube-like atrium, which is only slightly widened at its upper end. Under the light microscope, the whitish thin-walled atrium is clearly distinguishable from the black solid bulge.

Cross-sections of the respiratory suture have not been made, but significant differences to other *Eupaulianina* are unlikely. The ecdysial tube is very short. Its internal opening is hidden in a deep crease of the atrial wall near the border between its upper and lower sections, which is marked by a rounded transverse fold projecting into the lumen of the atrium (Figs 61, 62). The spongy chitinous coat on the inside walls of the atrium (Figs 63, 64) differs little from the analogous structures of *P. rivalis*.

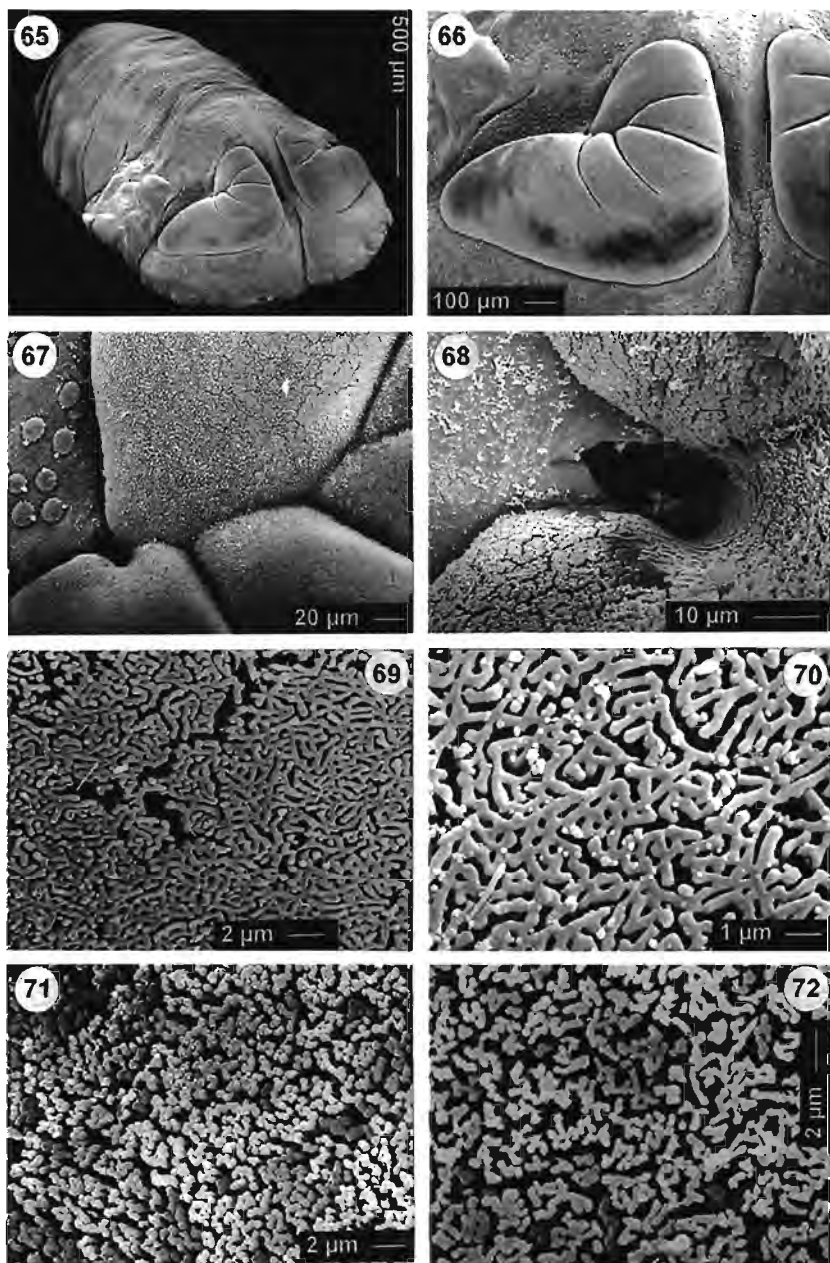
The granules on the abdominal tergites are cupola-shaped, with a terminal blister and a group of spines (Figs 98–100). On the thorax, the granules are flattened and most of them resemble a sharp-edged coin. The group of spines is missing here. The size of the granules varies between 15 and 20 μm .

P. (E.) alexanderi Stuckenberg, 1958

Length of pupae: 4.93 mm; 5.25 mm (Stuckenberg 1958). Examined specimen: 4.5 mm.

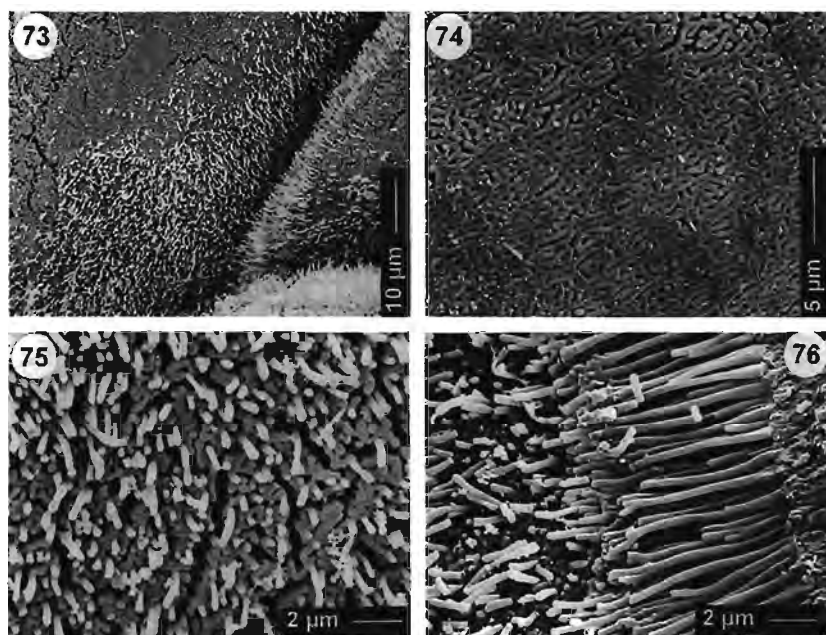
The pupae of this species are broader and more flattened than Fig. 65 suggests, because of the artificial shrinkage of the examined specimen; this shrinkage has however affected the abdomen to a greater extent than the thorax. The gills are large and occupy, in the form of 2 bilobed, slightly vaulted processes, almost all the posterior half of the pronotum (Figs 65, 66). Four narrow fissures divide the equally rounded surface of the gills into 5 raised areas. The large elevation 1 forms the outside half of the gill; the other 4 cover the strongly bent inner part. Fissures 1 and 2 are short and barely extend over the ridge of the respiratory process; however, the 3rd and 4th fissures also do not reach the anterior margin of the gill. All fissures arise from a short transverse trench (Fig. 67), along the base of which the respiratory suture reaches the 3rd fissure, as seen in the previous species. In contrast to *P. silva*, the 3rd fissure is underpinned over quite a long distance by a (using light microscopy, whitish coloured) elongation of the atrium (Fig. 77), although branches of the respiratory suture are also absent in *P. alexanderi*. The ecdysial pore is located in a small but distinct notch in the corner of the angled posterior margin of the gill (Figs 66–68).

The structure of the plastron varies considerably and differs in some characters from the other examined *Paulianina* species. The large elevation 1, except for its descending innermost part, is covered with a highly regular hair-carpet plastron formed by individual, densely packed trichomes (Fig. 71). However, near the first furrow, this plastron type changes rapidly into another plastron structure that is characterised by irregular anastomoses between the apices of the trichomes (cf. Figs 69, 70, 72). This latter plastron type or variants of it, also occurs on all surfaces of the



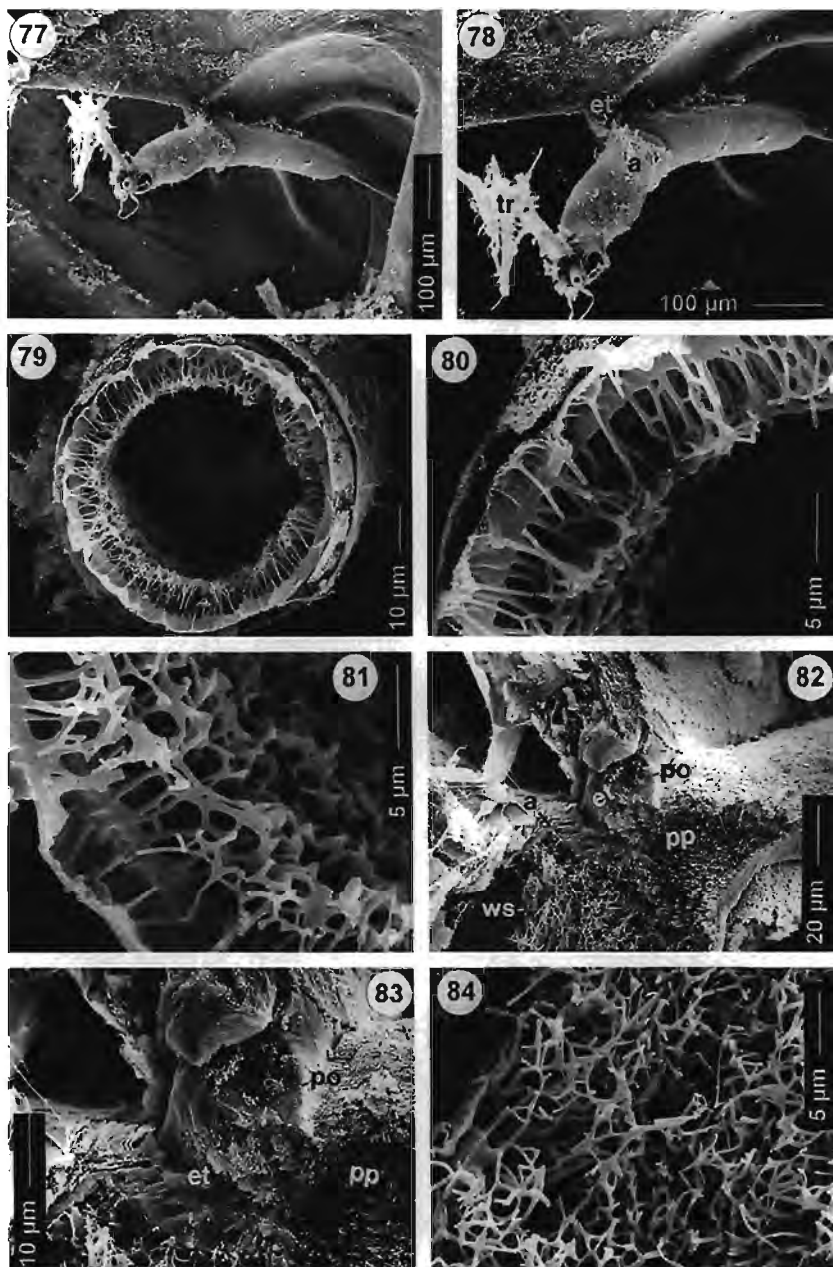
Figs 65–72. *Paulianina (E.) alexanderi*, external gill structures. 65. Pupa, fronto-lateral view. 66. Right gill. 67. Notch at the posterior margin of the gill, and the central part of the system of furrows between the respiratory elevations. 68. Ecdysial pore in the posterior notch of the gill; a long diatom lies above the opening. 69–70. Plastron structure on middle levels of the respiratory elevations; examples taken from the 4th and 3rd elevations. 71. Plastron structure on the dorsal region of the four inner elevations; example taken from elevation 5. 72. Typical plastron structure on the large 1st elevation.

inner elevations. The anastomoses are always predominantly shaped as meandering ramifying bands, so that the surface pattern of the plastron resembles more a labyrinth than a network with small round aeropyles, even on areas with a high density of anastomoses (Fig. 74). On the upper region of the 4 inner elevations (in particular on elevation 5), relatively few trichomes are apically fused with one another (Fig. 72), but the number of anastomoses increases towards lower levels of the gill (Figs 69, 70, 74). This trend ends near the ground of the fissures, where shortening of the anastomoses and dismemberment of the labyrinthine plastron surface are found; as a further structural modification, numerous filamentous outgrowths arise vertically from the anastomoses, so that the plastron appears to be overgrown by epizoid algae (Figs 67, 73, 75). Cross-sections of these lower regions of the plastron (Fig. 76) demonstrate that the trichomes of this area vary in length, and most of the peculiar filamentous outgrowths may therefore be the apices of trichomes projecting over the level of anastomoses.



Figs 73–76. *Paulianina* (*E.*) *alexanderi*, plastron structure. 73. Region of the fork at the posterior end of the 4th elevation, showing the transition from a plastron type with a well-developed cover membrane to the 'pubescent' plastron surface on the lower levels of the gill. 74. Detail: plastron type with a well-developed cover membrane. 75. Detail: plastron region with a 'pubescent' surface. 76. Section through this plastron type in the ground of the 3rd fissure, taken so that the left part of the figure shows the plastron surface in dorsal view.

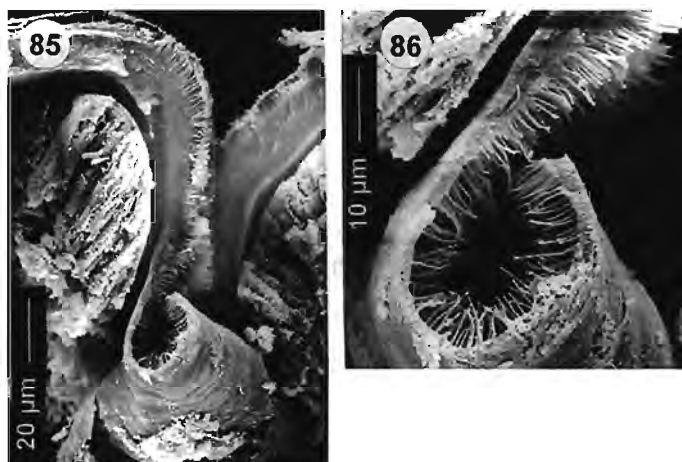
The atrium has a length of about 400 µm, and its horizontal upper part differs little in size and diameter (70–90 µm) from the oblique lower half (Figs 77, 78). The fold between the 2 sections is well marked. The inner walls of most regions of the atrium are coated with a low, but intensely interconnected carpet of branched trichomes, which clearly delimits a wide central hollow channel (Figs 79–81, 84). It is



Figs 77–84. *Paulianina (E.) alexanderi*, structure of atrium. 77–78. View of the undersurface of the left gill, showing the atrium (a), ecdysial tube (et) and branches of the trachea (tr). 79. Section through the atrium ventral to the level of the internal opening of the ecdysial tube. 80–81. Details: wall structures of the atrium in this region. 82. View (after removal of the right part) of the left wall of the ecdysial tube and the underlying atrium; po = edge of the ecdysial pore, et = wall of the ecdysial tube, a = upper border of the atrium, ws = wall structures of the atrium, pp = 'pubescent' plastron surface near the base of the furrow in front of the notch (compare with Fig. 67). 83. Detail, showing the ecdysial tube at higher magnification. 84. Detail: wall structures of the atrium.

noticeable that the threads of the cuticular network are often expanded to flat sheets (Figs 81, 84). The coat on the wall inside the horizontal part of the atrium is composed mainly of individual hair-like trichomes (Figs 85, 86). The respiratory suture is obstructed in the same manner as in the other species, but is not clearly demarcated from the long contact zone of the opposite side walls of the respiratory elevations, that descend almost perpendicularly in their lower region (Fig. 85). The ecdysial tube (Figs 82, 83) is extremely short and has a length less than 30 μm .

The granules of *P. alexanderi* are also special. They are round in contour and have a slightly vaulted dorsal face that is separated by an undulating edge from the narrow marginal region (Figs 101–103). The base of the granules is well demarcated from the surrounding integument. The small terminal blister inserts higher than in other *Eupaulianina* species, within the undulating edge. A group of spines is present on the abdominal granules. Their number reaches 10 on the middle region of the abdominal tergites, but decreases on the marginal regions and towards the apex of the pupa. The diameter of the granules is approximately 15 μm .

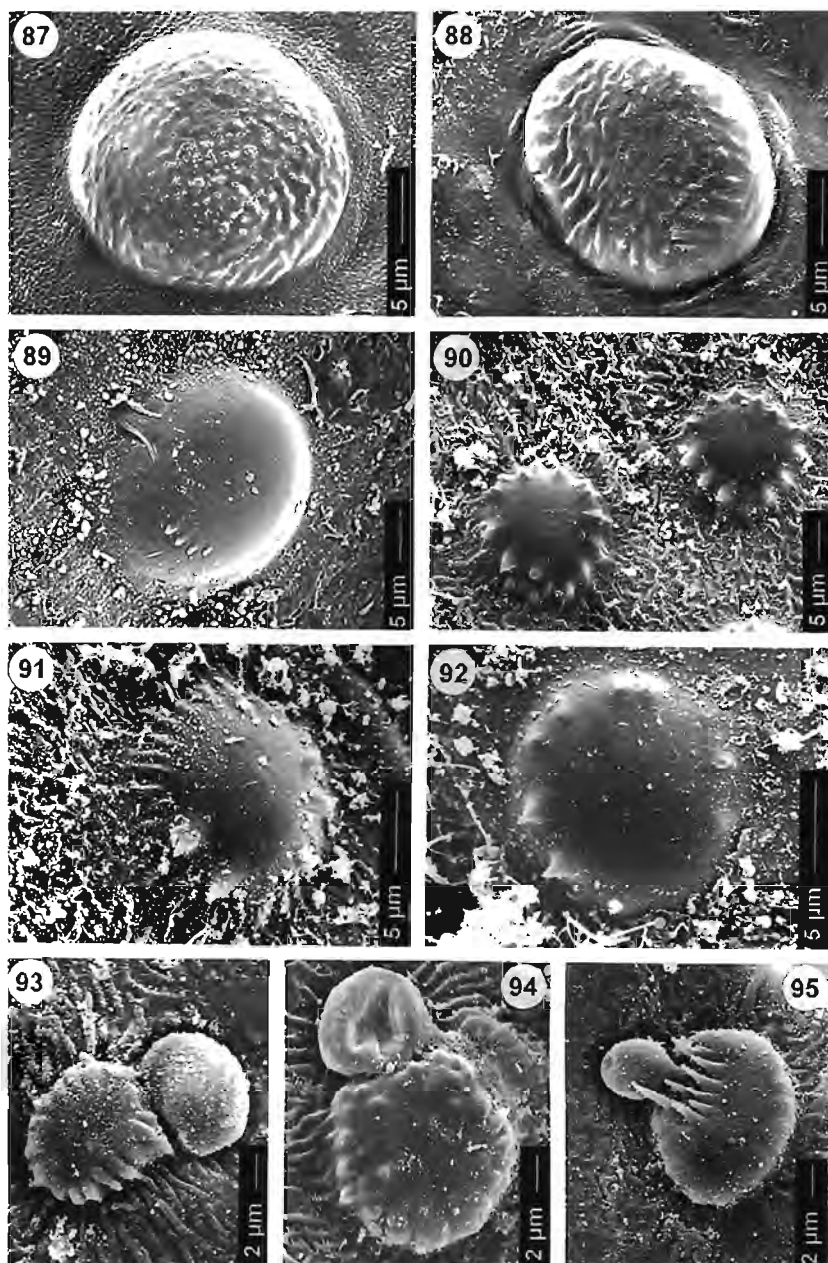


Figs 85–86. *Paulianina* (*E.*) *alexanderi*, structure of atrium. 85. Section through the 3rd fissure of the gill and the horizontal branch of the underlying atrium (compare with Figs 77–78); a part of the right wall of the suture is broken off. 86. Detail: wall structures inside the atrium and the respiratory suture; the right wall of the suture has been removed.

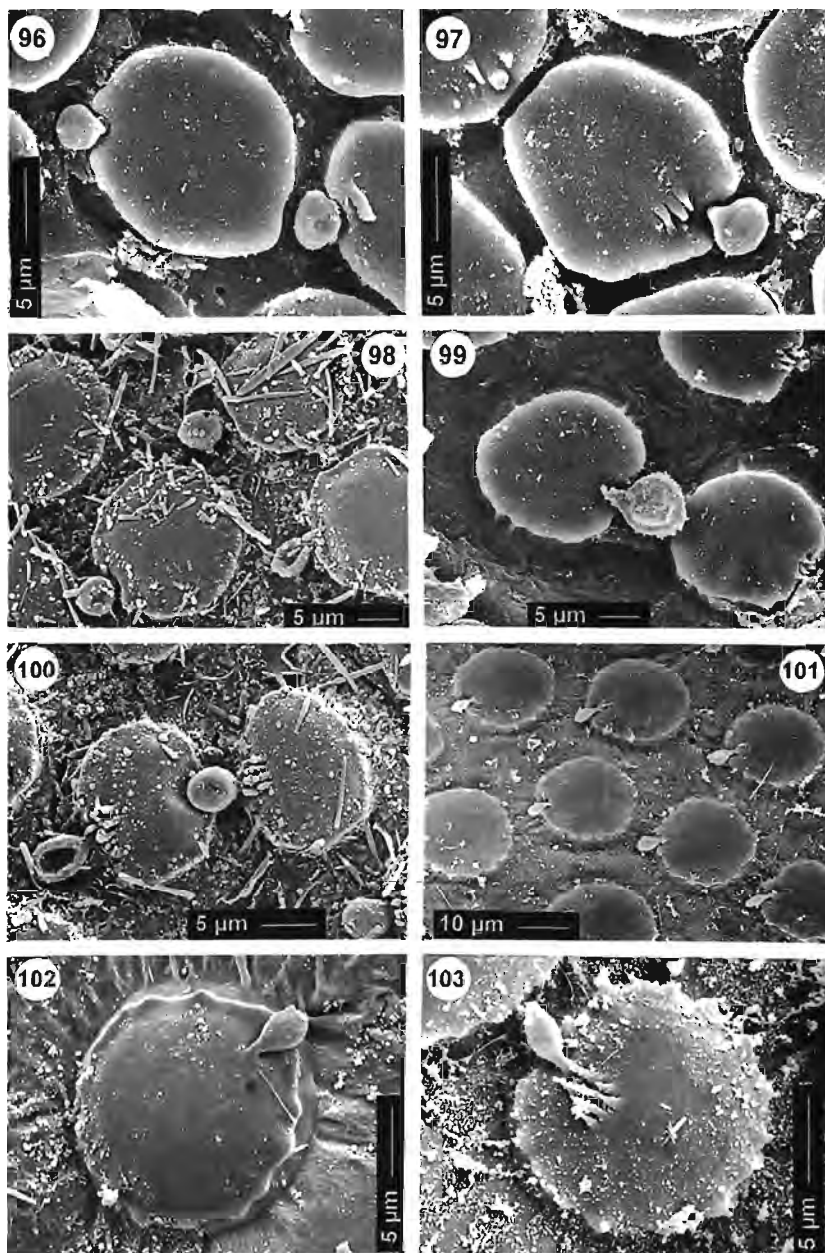
DISCUSSION

Phylogenetic considerations

In his monograph about the Australian Blephariceridae, Zwick (1977) thoroughly discussed the phylogenetic relationships within the Edwardsininae, and has in some cases given, based on his substantially expanded knowledge of the subfamily, a new interpretation of the extensive information on *Paulianina* given by Stuckenberg (1958). The Edwardsininae are the sister-group of the Blepharicerinae, and there can also be no doubt that the two genera of the Edwardsininae are monophyletic taxa (Fig. 104). The relationships within these genera are, however, less clear, at least to date. Whereas the Australian *Edwardsina* obviously form a monophyletic species



Figs 87–95. Granules of *Paulianina* species. 87–89. *Paulianina* (*P.*) *ingens*. 87–88. On the prothorax; macerated. 89. On the lateral region of the 6th abdominal tergite; the band-like structure at the upper margin is usually lacking. 90–92. *Paulianina* (*P.*) *umbra*; macerated. 90. On the 6th abdominal tergite. 91. On the anterior intersegmental region of the 5th abdominal tergite. 92. On the lateral posterior region of the 4th abdominal tergite. 93–95. *Paulianina* (*E.*) *pamela*. 93. Prothoracic granule. 94. On the prothorax in front of the gills; blister collapsed. 95. On the 4th abdominal tergite.



Figs 96–103. Granules of *Paulianina* species. 96–97. *Paulianina* (*E.*) *rivalis*; macerated. 96. On the lateral portion of the 1st abdominal tergite. 97. On the 5th abdominal tergite. 98–100. *Paulianina* (*E.*) *silva*. 98. On the terminal tergite of the abdomen. 99. On the lateral portion of the 2nd abdominal tergite; macerated. 100. Near the middle of the 3rd abdominal tergite. 101–103. *Paulianina* (*E.*) *alexanderi*. 101. On the terminal tergite of the abdomen. 102. Prothoracic granule in front of the gill. 103. On the penultimate abdominal tergite.

group within the subgenus *Tonnoirina* (Zwick 1977; Arens 1998), a sound phylogenetic interpretation of the South American *Edwardsina* is still impossible because of our limited knowledge of this faunal entity, which contains all known species of *Edwardsina* s.s. and some members of *Tonnoirina*.

The division of *Paulianina* into the two subgenera *Paulianina* s.s. and *Eupaulianina* was previously made by Stuckenberg (1958). The monophyletic nature of *Eupaulianina* is well documented on the basis of several apomorphic characters, and has been confirmed by my investigation of the fine structure of the pupal gills and granules. Nevertheless, I have my doubts about whether *Paulianina* s.s. is also a monophyletic taxon. Most diagnostic characters that Stuckenberg (1958) found suitable to distinguish species of *Paulianina* s.s. from those of *Eupaulianina* are plesiomorphic (see Zwick 1977: 25). The subgenus is at present defined only by three apomorphies, two of which (not only L4, but also L3 with bisegmented antennae; adults with only four segments on maxillary palpus) are less convincing. However, the reduced labium and the unusual shape of the mandible in the larvae of *Paulianina* s.s. (Stuckenberg 1958; Zwick 1977) are strong arguments for the monophyly of the subgenus. Nevertheless, my investigation of the fine structure of the pupal gills leads me to propose an alternative phylogeny, in which *Paulianina* s.s. is interpreted as a paraphyletic taxon (Fig. 104).

Among the known *Paulianina* species, *P. ingens* has the most primitive pupal gill structure. Zwick (1977) previously emphasised that there are striking similarities between this species and some South American *Edwardsina*. This applies not only to the topography of the lamellae (oriented for the most part parallel to the body axis), the posterior notch of the baseplate (behind the 2nd lamella), and the respiratory suture (between the notch and the 3rd interlamellar space), but also to the shape of the respiratory processes (lamellae) and their number (always 9). An even more intriguing finding is the striking congruence of the complex course of the respiratory suture in *Paulianina ingens* (Fig. 2) and *Edwardsina chilota* (Arens 1995: 2326). Furthermore, with respect to the shape and size of the posterior notch, *P. ingens* resembles this South American species more than the other *Paulianina* species. One can therefore postulate that these gill features and the complex course of the suture are primitive throughout the subfamily (see also Arens 1995).

Substantial evidence indicates that *P. umbra* is the 'next of kin' of *P. ingens*, at least among the *Paulianina* species with known pupae. Its gills are differentiated into 9 longitudinal processes, and the atrium and most regions of the plastron are similar in structure to the respective organs of *P. ingens*. Other gill characters of *P. umbra*, however, appear to be derived, and are also found in a similar version in the species of *Eupaulianina*: (1) at least one of the respiratory processes is transformed into a blunt ridge, (2) the surface of this bulge is coated with a hair-carpet plastron (with the exception of the region near the 1st interlamellar space), (3) the course of the respiratory suture is simple and sigmoid, (4) the suture ends on the posterior third of the 3rd interlamellar space, and (5) the notch at the posterior margin of the gill is comparatively short.

The *Eupaulianina* species undoubtedly form a monophyletic taxon. In addition to the reduced number of respiratory processes and other convincing synapomorphies (Stuckenberg 1958; Zwick 1977), the pupae of this subgenus are distinguished from

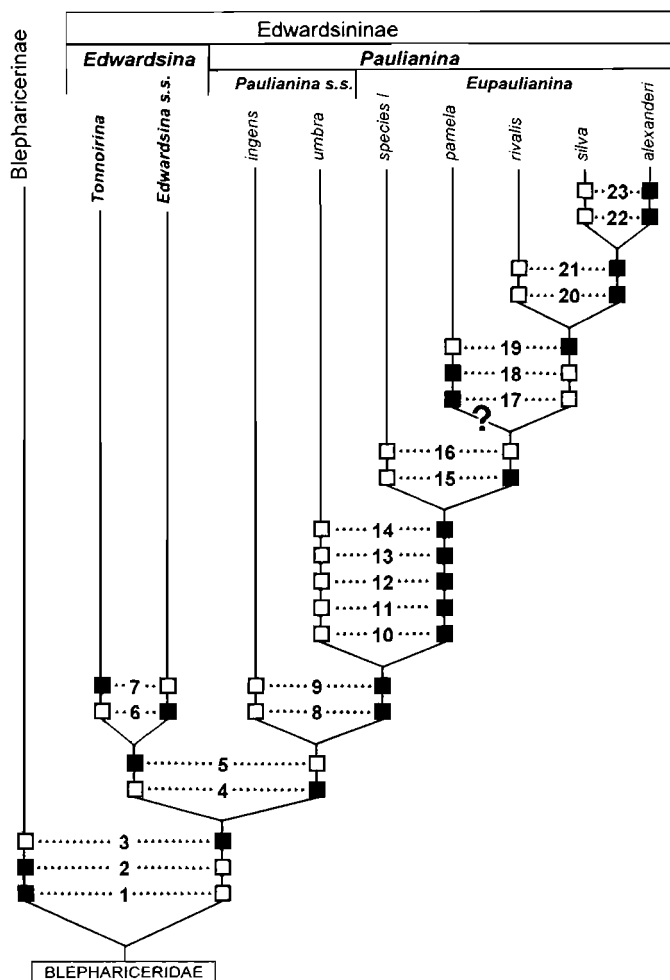


Fig. 104. Phylogenetic relationships between the examined species of *Paulianina* and related taxa. Black squares: apomorphic character. White squares: plesiomorphic character (given in parentheses). 1, Pupal spiracle with atrial lid and four circumspiracular lamellae (pupal spiracular gills composed of a baseplate and 9 longitudinal lamellae; ecdysial pore outside the baseplate). 2–7, See Zwick (1977: 25), characters 1–17, 28–34. 8, Outermost respiratory process enlarged and transformed into a blunt ridge coated with a hair-carpet plastron (all processes shaped as lamellae and coated with a plastron with cover membrane). 9, Course of respiratory suture simple and sigmoid; suture comparatively short (course of suture complex; suture long). 10, Number of respiratory processes reduced to 6 or 5. 11, Primary plastron structure completely replaced by other types of plastron structure. 12, Spongy chitinous coat on inner walls of pupal atrium reduced. 13, Granules on pupal tergites with terminal blister. 14, See Zwick (1977: 25), characters 18–21, 23–24. 15, All respiratory processes transformed into blunt elevations or broad swellings. 16, Cover membrane of plastron almost completely reduced. 17, Innermost respiratory process wider than the others, with a transverse rim arising from its posterior end. 18, Plastron structure modified, with a tessellated surface caused by anastomoses between groups of trichomes. 19, Topography of gills modified and highly typical. 20, Ventral plate of adult male with a median tube between the parameres (see Stuckenberg 1958: 101). 21, All respiratory processes shaped as broad swellings separated by narrow fissures. 22, Plastron structure modified, with unique structural details. 23, Respiratory suture lengthened so that 3rd interlamellar space is for the most part underpinned by the atrium (atrium reaches only the posterior region of this space).

all other blepharicerid pupae, as far as I can tell, by the small blister of the granules. It is to be expected that the highly interesting *P. (E.) species I* Stuckenberg, 1958 (Fig. 105), which in some aspects seems to be an intermediate form between *P. umbra* and the other *Eupaulianina* species, also has such blisters. The general appearance of its gills is similar to those of *P. umbra* (inner processes shaped as lamellae, the outermost process transformed into a blunt ridge and with a posterior elongation interrupted by the notch), but there are only 6 processes and the second is expanded into a hump. In the four *Eupaulianina* species that I have examined, the respiratory processes are all modified into hump-like elevations or broad swellings; the plastron type with a regular cover membrane, which is most common in blepharicerid pupae (Arens 1995 1998), is never found in *Eupaulianina* pupae, despite their various plastron modifications.

Paulianina pamela obviously has an isolated systematic position within this subgenus. Its respiratory processes are relatively less expanded and have proportions different from the elevations of the other *Eupaulianina*; moreover, its plastron structure is special in some details, in spite of an obvious similarity in general appearance to the related species. The transverse ridge along the posterior margin of the baseplate resembles a convergently evolved structure of *P. umbra* (and *P. species I*), but arises from the innermost elevation instead of the outermost.

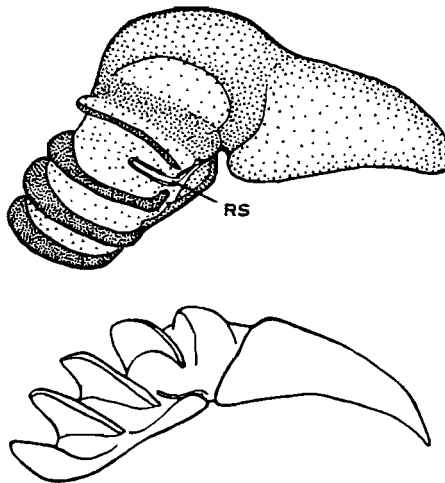


Fig. 105. Right gill of *Paulianina species I* in dorsal and posterior view (from Stuckenberg 1958).

The other three species: *P. rivalis*, *P. silva* and *P. alexanderi*, are certainly closely related to each other. *P. rivalis* and *P. silva* agree in nearly all details of their gill structure, apart from the respiratory swellings of *P. silva* being more expanded and its 3rd interlamellar space being extremely deep. *P. alexanderi* has a plastron structure unique among *Paulianina*, although the topography of its gills indicates a close relationship to *E. silva*. In addition, Stuckenberg (1958) found correspondence with respect to other synapomorphic characters between these two species, and thus he defined them as the *silva*-group within the subgenus *Eupaulianina*. I agree with Stuckenberg, even if the modified plastron structure, the unusual shape of the atrium,

and the special granules, are evidence of the more distant systematic position of *P. alexanderi* from *E. rivalis*.

Knowledge of the pupal gill structure of the other *Paulianina* species, especially that of *Paulianina hova* (*Paulianina s.s.*), would be of particular interest, but unfortunately only one adult female of this species has been found to date (Alexander 1952; Stuckenberg 1958). In addition, a comparative investigation of the fine structure of the larval mouthparts of *Paulianina* could decide the question regarding which of two similar features are the result of convergent evolution: the modified structure of the labium and mandible in *P. ingens* and *P. umbra*, as I suggest, or the correspondence in gill shape between *P. umbra* and some *Eupaulianina*, as Stuckenberg and Zwick assume.

Variation of plastron structure and functional aspects

My present and former studies (Arens 1995 1998) have shown that there is a considerable diversity in plastron structure among blepharicerid pupae. Two types of plastron structure, the 'plastron with cover membrane' and the 'hair-carpet plastron' (Arens 1995), are the most common, so that retention of this terminology is appropriate, even if the variety of plastron structure among the Edwardsininae is greater than I foresaw at the time of this definition. In both the Madagascan and the Australian faunal entities of the Edwardsininae, not only the gill shape, but also the primary plastron structure, are transformed during the course of evolution. It is interesting with regard to taxonomy and phylogeny that, despite this manifold variation and some cases of convergence, the plastron structure is often special and constant enough to be a characteristic feature of a group of related species. Foam-like plastron elements, for example, are found only on the gills of the *williamsi* and *torrentium* subgroups within the Australian *Edwardsina* (Arens 1998).

The plastron with a regular cover membrane seems to be the primitive plastron structure in blepharicerid pupae (Arens 1995). The present findings relating to *P. ingens* corroborate this hypothesis. Among the Edwardsininae, this plastron type is found, in particular, in species constituting relatively early branches of the phylogenetic tree (e.g. *Edwardsina polymorpha*, *E. gigantea*, *Paulianina ingens*, *P. umbra*), but is also present in many Blepharicerinae (e.g. *Liponeura*, *Hapalothrix*, *Paltostoma*, *Elporia*). In *Paulianina*, there is obviously a correlation between the expansion of the respiratory processes and the modification of this plastron type by the reduction of the cover membrane. Thus, the eight lamellae of *P. umbra* are still coated with the primary plastron type, whereas its enlarged outermost process bears the same hair-carpet plastron as is also present, in some variants on the inner regions of the gills, on the respiratory swellings of all examined *Eupaulianina* species.

The peculiar plastron surface of the four inner respiratory elevations of *P. alexanderi* is certainly a further step of modification, but, at the same time, an example of the striking convergences in plastron structure for which one has to be prepared. The South American *Edwardsina chilota* has an almost identical plastron structure, characterised by a cover membrane which is composed of meandering threads or strongly reduced or overtopped by filamentous outgrowths (compare Figs

72, 70, 73 with Arens 1995: Figs 33, 34, 37). There is no doubt that this astonishing resemblance is the result of convergent evolution. Further examples of this phenomenon are the tipulids *Dicranomyia trifilamentosa* and *Geranomyia unicolor* (Hinton 1968: 129, 128), the plastrons of which are in some aspects the very image of Figs 11 & 60 in my present study. It remains to be seen whether the primary plastron structure, which is preserved at least in *E. gracilis* (Hinton 1968: 140), is also modified in other South American *Edwardsina*.

One could assume that the loss of the regular cover membrane in the *Eupaulianina* species is involved with the flattening of the respiratory processes and their reduced exposure to the flow; however, the presence of a hair-carpet plastron on the tall lamellae in some Apistomyiini (subfamily Blepharicerinae), and the plastron structure of *E. chilota*, speak against this speculation. Furthermore, I cannot see a plausible reason for the transformation of the respiratory processes into broad swellings. According to the observations of Stuckenberg (1958), all *Paulianina* species have similar life cycles (poly-generative summer-type; Kitakami 1950) and occur in the same type of habitat on rock faces under a strong flow of water (submerged type), except *P. umbra*, which develops on steep rock faces on waterfalls (cascade-type). *P. ingens*, *P. silva* and *P. alexanderi* may even inhabit the same locality. *P. pamela* is also found, sometimes mixed with *E. rivalis*, on steep surfaces over which a thin film of water runs. Thus, the same phenomenon has to be stated as discussed in my study of the Australian *Edwardsina* (Arens 1998): the amazing diversity in gill structure seems not to be correlated with differing ecological demands of the species.

The granules

A comparative investigation of these tiny dark outgrowths has not previously been made, although some interesting taxonomic and phylogenetic information might be expected from an SEM analysis of their fine structure, and analogous granules occur in other aquatic insects living in fast-flowing streams. In *Elmis* larvae (Coleoptera, Elmidae), the tergites of the thorax and abdomen bear oval, ca. 20 µm long integumental structures with a granulated surface, and a terminal appendix shaped like a tuft of threads arising from a short stem (Wichard *et al.* 1995). The dorsal surface of the torrenticolous larvae of *Psephenus* (Coleoptera, Psephenidae) is adorned by three types of outgrowths: round, ca. 15 µm long humps with a roughly granulated surface; ca. 12 µm structures with strongly dentate edges; and small, only 7 µm long balls surrounded by a collar with undulating edges (Wichard *et al.* 1995). The presence of similar outgrowths in different groups of stream-living insects indicates that they may be an adaptation to a special environmental factor of this habitat. The characteristic pattern of arrangement of the granules in *Eupaulianina* leads me to hypothesise that the flow of water is this factor, because the flow pattern around blepharicerid pupae (Pommen & Craig 1995) agrees remarkably well with the varying orientation of the granules. As a result of the attachment of blepharicerid pupae in a rheonegative position on rocky surfaces, the water flow passes from the rear along the abdominal tergites. Downstream of the pupae, a region of low pressure

is produced, with the consequence that a lateral thoracic flow (LTF in Pommen & Craig 1995) passes diagonally from the region of the pterothecae into the gap between the gills. Additionally, water flows around the base of the gills (RV), and two vortices downstream of the pupae (PV) force an outward movement of the water directly anterior of the gills. All in all, a considerable correspondence between the flow pattern and the orientation of the granules is found, and even the irregular pattern of arrangement of the granules on the apex of abdomen may be explained by a disturbed flow pattern that one has to expect in this region of ram pressure. It is therefore likely that the granules have the function to modify the hydrodynamic characteristics of the pupae. Moreover, the terminal blisters of the granules of *Eupaulianina* certainly have only a mechanical function, because some peculiarities of the development of Diptera (Hinton 1968 1976; Arens 1995) rule out that they serve as chloride cells, as do other thin-walled outgrowths of some hemimetabolous aquatic insects (Wichard *et al.* 1972; Zwick 1980; Wichard *et al.* 1995). It would be worthwhile to search for further examples of convergently evolved granules, and to check whether a characteristic pattern of their orientation is found in immobile stream insects other than blepharicerid pupae.

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